Considered /Wu-Cheng Winston Shen/ 08/10/2009

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	D'Amour et al.
App. No	:	10/584,338
Filed	:	January 9, 2007
For	:	DEFINITIVE ENDODERM
Examiner	:	Wu Cheng Winston Shen
Art Unit	:	1632
Conf No.	:	3658

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF
DR. KEVIN D'AMOUR UNDER 37 C.F.R. § 1.132

Sir:

I, Dr. Kevin D'Amour, do hereby declare and state that:

1. I am a co-inventor of the subject matter described and claimed in U.S. Patent Application Serial No. 10/584,338, filed on January 9, 2007 entitled, "DEFINITIVE ENDODERM".

2. I am a Principle Scientist at CyThera Corporation, a wholly owned subsidiary of Novocell, Inc., which has an ownership interest in the above-identified patent application. I have been working in the field of developmental biology, and more specifically, stem cell research for more than 10 years. My *curriculum vitae* is attached as Exhibit B.

3. I am familiar with the prosecution history of Patent Application Serial No. 10/584,338, including the subject matter of the currently pending claims.

4. I understand that the Examiner rejected the claims, in part, for allegedly being non-enabling under 35 U.S.C. § 112, first paragraph. According to the Examiner,

the specification allegedly does not teach "providing said cell population with a TGFβ superfamily growth factor and a Wnt-pathway activator" to generate definitive endoderm from pluripotent human cells.

5. In contrast to the Examiner's assertion that only Activin A has been described to generate definitive endoderm from pluripotent human cells we demonstrate in detail below that other TFG-β superfamily growth factors besides Activin A can be used to induce definitive endoderm formation from human embryonic stem (hES) cells.

6. I performed the following experiments with the assistance of others. These data are also similarly described in Example 1, Figure 2 of U.S. Serial Number 12/132,437, entitled "GROWTH FACTORS FOR PRODUCTION OF DEFINITIVE ENDODERM," filed June 3, 2008:

> Undifferentiated hES cell culture plates were differentiated in RPMI (Mediatech) supplemented with Glutamax, penicillin/streptomycin, and with either: i) 100 ng/mL Activin A, ii) 100 ng/mL of Activin B (R&D Systems), iii) 100 ng/mL of GDF8, or iv) 100 ng/mL of GDF11 (R&D Systems) and varying concentrations of defined FBS (HyClone). Additionally, 0.1% BSA (Invitrogen/Gibco) and 75 ng/mL Wnt3a (R&D Systems) were added on the first day (d0) of differentiation. FBS concentrations were 0% for the first 24 hour and 0.2% for the second 24 hour. Replicate samples were collected from each culture condition at 0, 24 and 48 hours after differentiation commenced and analyzed for relative gene expression using real-time quantitative PCR.
>
> As shown in Exhibit A, Figure 1, Activin A, Activin B, GDF8 and/or GDF11 can induce definitive endoderm differentiation from hES cells. The addition of 100 ng/ml Activin A resulted in a 1392-fold induction of SOX17 (FIG. 1C) and 197-fold induction of FOXA2 (FIG. 1D) gene expression, as compared to undifferentiated hESCs. The addition of 100 ng/ml Activin B resulted in a 2072-fold induction of SOX17 (FIG. 1C) and 66-fold induction of FOXA2 gene expression, as compared to undifferentiated hESCs. The addition of 100 ng/ml of GDF8 resulted in a 1324-fold induction of SOX17 (FIG.1C) and 438-fold induction of FOXA2 (FIG.1D) gene expression, as compared to undifferentiated hESCs. The addition of 100 ng/ml of GDF11 resulted in a 1306-fold induction of SOX17 (FIG.1C) and a 382-fold induction of FOXA2 (FIG.1D) by day 2 of differentiation, as compared to undifferentiated hESCs. In addition, the differentiation to definitive endoderm proceeded through a mesendoderm intermediate as evidenced by the transient brachyury expression witnessed at 24 hours (FIG.1A). The lack of induction of SOX7 (FIG.1E) gene expression indicated that the endoderm lineage produced was definitive endoderm and not extra-embryonic endoderm, which does appreciably express SOX7.

7. The data in Sections 6 and 7 above and accompanying Figures in Exhibit A demonstrate that in addition to Activin A, other TGF-β superfamily members, *e.g.*, Activin B, GDF8 and/or GDF11, are efficient in generating definitive endoderm cell populations from hES cells.

8. In addition to the experiments described in item 6, I performed the following experiments with the assistance of others. These data are also similarly described in Example 3, Figures 4-5 of U.S. Serial Number 12/132,437, entitled "Growth factors for production of definitive endoderm," filed June 3, 2008:

> Undifferentiated human embryonic stem (hES) cells (CyT49; passage 48) were maintained substantially as described in Example 1 above. Seven hES cell culture plates were then differentiated in RPMI (Mediatech) supplemented with Glutamax, penicillin/streptomycin, and either: i) activin A (100 ng/ml), ii) TGF-beta1 (100 ng/ml), iii) TGF-beta3 (100 ng/mL), iv) TGF-beta2 (200 ng/ml), v) GDF3 (500 ng/ml), vi) GDF9 (4 ug/ml), or vii) GDF15 (2.5 ug/ml) and varying concentrations of defined FBS (HyClone). Additionally, 0.1% BSA (Invitrogen/Gibco) and 75ng/mL Wnt3a (R&D Systems) were added on the first day (d0) of differentiation. FBS concentrations were 0% for the first 24 h and 0.2% for the second 24 h. Replicate samples were collected from each culture condition at 0, 24 and 48 hours after differentiation commenced and analyzed for relative gene expression using real-time quantitative PCR.
>
> As shown in Exhibit A, Fig. 2, definitive endoderm was produced only in the activin A treatment condition (A100) as indicated by the induction of SOX17 (FIG.2A) and CER1 (FIG.2B) gene expression. Conversely, treatment with any of the other TGF-beta family member ligands resulted in little to no induction of SOX17 or CER1 but instead resulted in substantially induced expression of CXD2 (FIG.4C) over both the hES and the definitive endoderm expression levels. The induction levels for CDX2 observed in non-activin treated plates (i.e. TGF-beta 1, 2, 3 or GDF3, GDF9 and GDF15) over that observed with the activin A treatment was in the range from 900-fold higher expression for GDF3 to 2,667-fold higher expression for TGF-beta3. These data indicated that the described TGF-beta family ligands were not effective for directing differentiation of hES cells to definitive endoderm, at least under conditions similar to those where activin A was effective.
>
> To further demonstrate that the described TGF-beta family ligands were not sufficient to produce effective definitive endoderm differentiation, the plates were subjected to conditions that promote efficient differentiation of definitive endoderm cells to pancreatic endoderm, and subsequently endocrine cells. As shown in Exhibit A. Figures 3A-D, pancreatic endoderm and endocrine cell types were produced only in the activin A treatment condition (A100) as indicated by the induction of PDX1, PTF1A, NKX2-2 and INS gene expression. Pancreatic endoderm or endocrine type cells had PDX1 expression levels ranging between 1413-fold and 14,497-fold higher in the presence of activin A, as compared to in the presence of TGF-beta1 and GDF15, respectively (FIG.3A). Similarly, the pancreatic endoderm or endocrine cells expressing endocrine markers NKX2-2 and INS were typically not detected in the non-activin A treated plates (FIG.3C-D). Stated another way, the activin A treated plates were 1200-fold or more higher in expression of these markers than the non-Activin A treated plates.

9. The data in Section 8 above and accompanying Figures in Exhibit A demonstrate that TGF-β 1, TGF-β 2, TGF-β 3, GDF3, GDF9 and GDF15, do not efficiently generate definitive endoderm cell populations from hES cells.

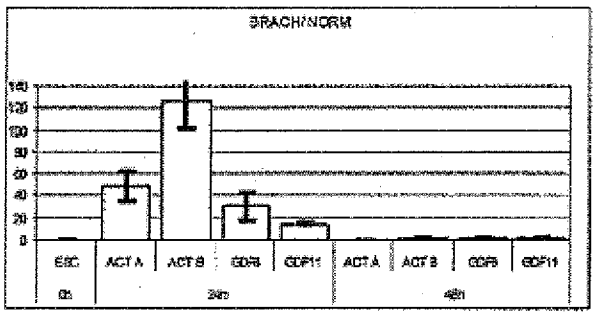
10. I further declare that all statements made herein of knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

May 12 2009
Date

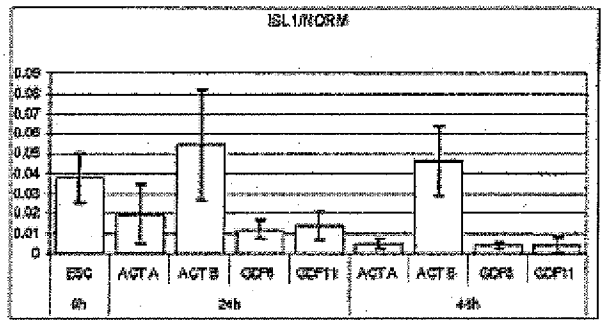
Kevin D'Amour, Ph.D.

EXHIBIT A

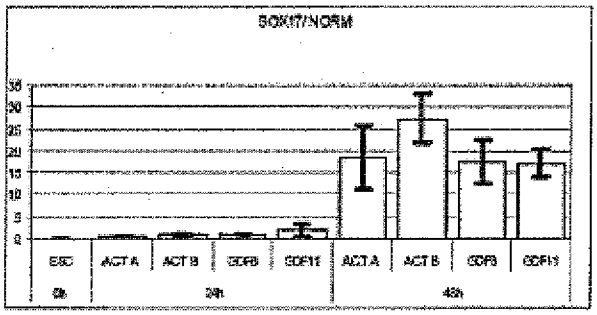
A



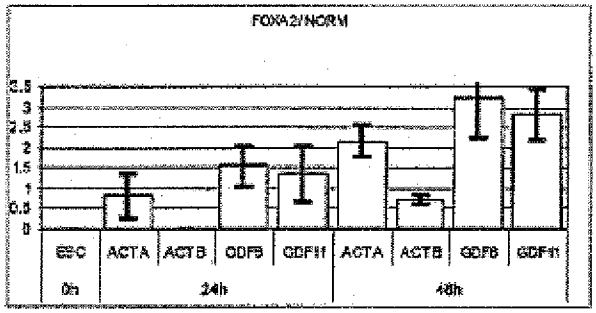
B



C



D



E

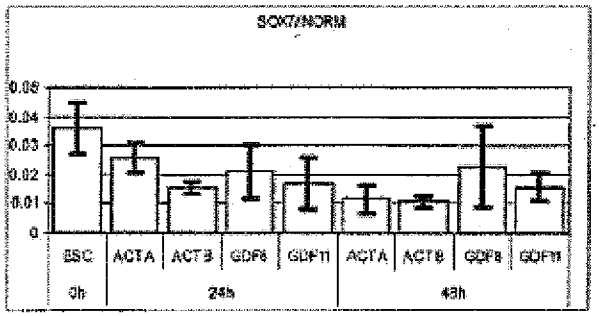
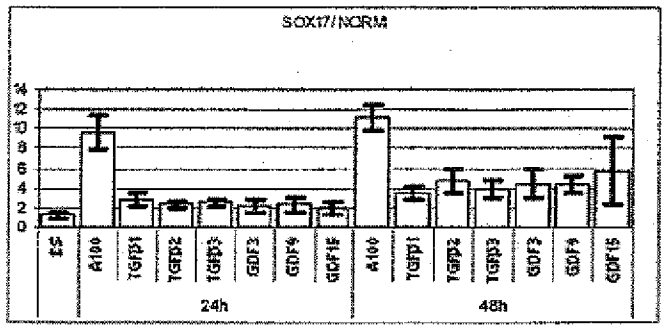
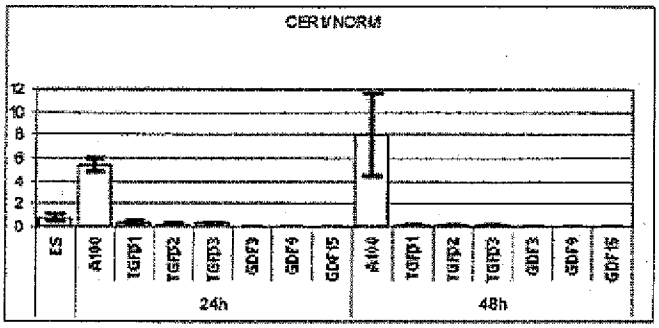


Figure 1

A


SOX17/NORM
ES
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
24h
48h

B


CER1/NORM
ES
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
24h
48h

C

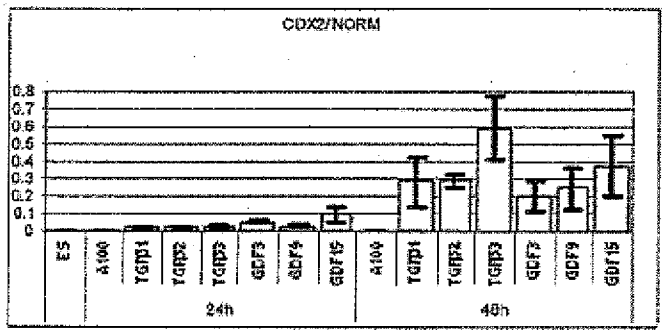

CDX2/NORM
ES
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
24h
48h

Figure 2

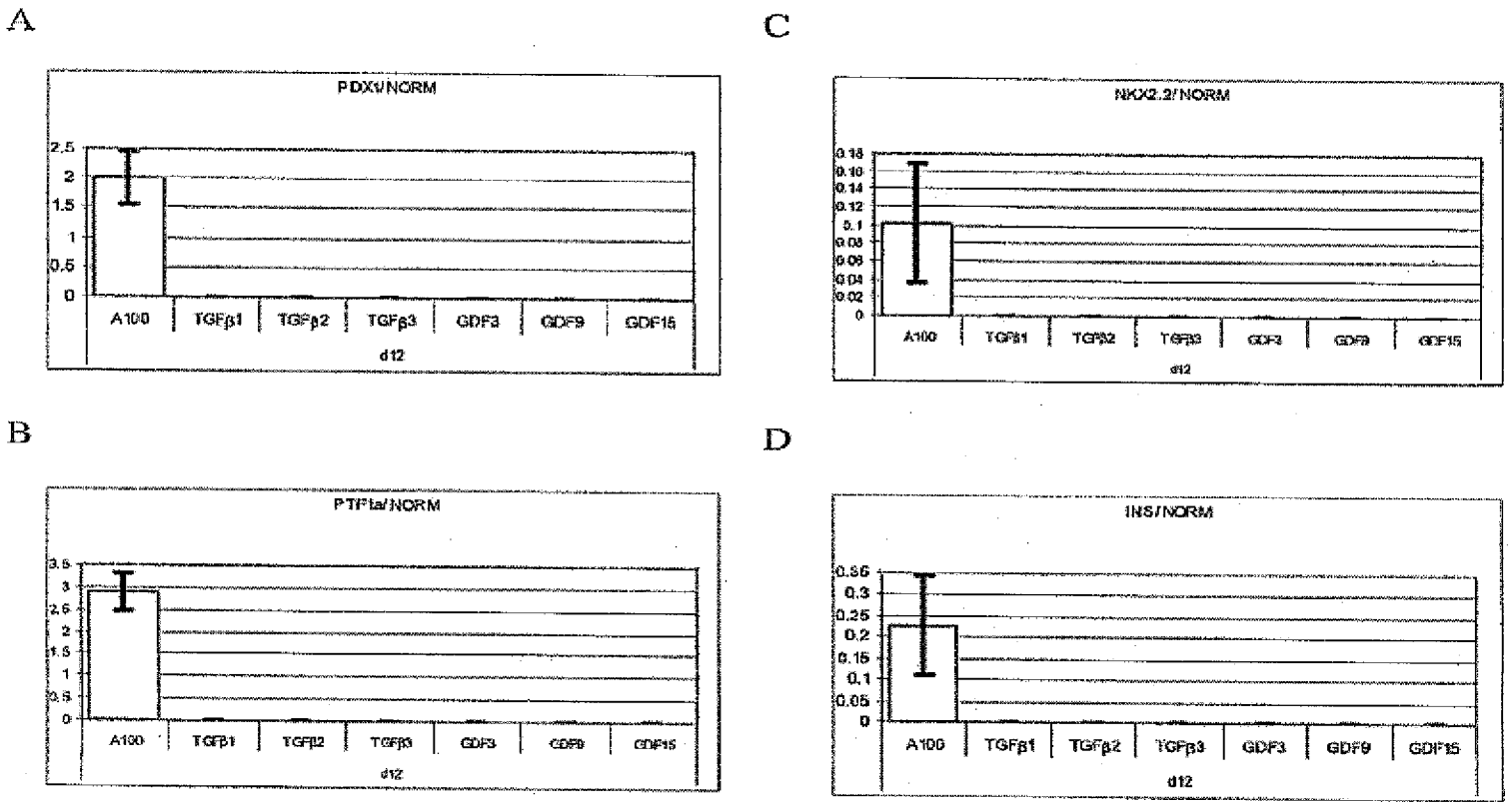

A
PDX1/NORM
2.5
2
1.5
1
0.5
0
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
d12
C
NKX2.2/NORM
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
d12
B
PTF1a/NORM
3.5
3
2.5
2
1.5
1
0.5
0
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
d12
D
INS/NORM
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
A100
TGFβ1
TGFβ2
TGFβ3
GDF3
GDF9
GDF15
d12

Figure 3

EXHIBIT B

Curriculum Vitae
Kevin A. D'Amour

Novocell, Inc.
3550 General Atomics Ct.
San Diego, CA 92121
www.novocell.com

(858)455-3581 (office)
kdamour@novocell.com

Education:

June 2002	Ph.D. in Biology	U. of California San Diego, La Jolla, CA
May 1994	B.S. in Animal Science	U. of New Hampshire, Durham, NH

Research Experience:

2/2008- Present	Principle Scientist, Novocell, Inc., San Diego CA
7/02-1/31/2007	Sr. Research Scientist, Novocell, Inc., San Diego CA
7/98-6/02	Graduate Student Researcher, UC San Diego, La Jolla CA Research Advisor: Dr. Fred Gage
8/97-6/98	Graduate Student Researcher, UC Berkeley, Berkeley CA Research Advisor: Dr. John Casida
1/95-7/97	Sr. Research Assistant, Gen-Probe Inc., San Diego CA
9/93-5/94	Undergraduate Fellow, U of New Hampshire, Durham NH Research Advisor: Dr. Dennis Bobilya

---

Publications:

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, **D'Amour KA**, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol (2008) Apr; 26(4):443-52.

Suh H, Consiglio A, Ray J, Sawai T, **D'Amour KA**, Gage FH. In Vivo Fate Analysis Reveals the Multipotent and Self-Renewal Capacities of Sox2(+) Neural Stem Cells in the Adult Hippocampus. Cell Stem Cell (2007) Nov 15; 1(5):515-528.

Wang L, Schulz TC, Sherrer ES, Dauphin DS, Shin S, Nelson AM, Ware CB, Zhan M, Song C-Z, Chen X, Brimble SN, McLean A, Galeano MJ, Uhl EW, **D'Amour KA**, Chesnut JD, Rao MS, Blau CA, and Robins AJ (2007) Self-renewal of human embryonic stem cells requires insulin-like growth factor-1 receptor and ERBB2 receptor signaling. Blood Dec;110(12):4111-9.

McLean AB, **D'Amour KA**, Jones KL, Krishnamoorthy M, Kulik MJ, Reynolds DM, Sheppard AM, Liu H, Xu Y, Baetge EE, Dalton S. (2007) Activin A efficiently specifies definitive endoderm from human embryonic stem cells only when PI3K signaling is suppressed. Stem Cells Jan; 25(1):29-38.

D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MC, Baetge EE. (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol Nov; 24(11):1392-401. Epub 2006 Oct 19.

D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol Dec; 23(12):1534-41. Epub 2005 Oct 28.

Wurmser AE, Nakashima K, Summers RG, Toni N, **D'Amour KA**, Lie DC, Gage FH. (2004) Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. Nature Jul 15; 430(6997):350-6.

Brandon EP, Mellott T, Pizzo DP, Coufal N, **D'Amour KA**, Gobeske K, Lortie M, Lopez-Coviella I, Berse B, Thal LJ, Gage FH, Blusztajn JK. (2004) Choline transporter 1 maintains cholinergic function in choline acetyltransferase haploinsufficiency. J Neurosci Jun 16; 24(24):5459-66.

D'Amour KA, Gage FH. (2003) Genetic and functional differences between multipotent neural and pluripotent embryonic stem cells. Proc Natl Acad Sci U S A Sep 30; 100 Suppl 1:11866-72.

Brandon EP, Lin W, **D'Amour KA**, Pizzo DP, Dominguez B, Sugiura Y, Thode S, Ko CP, Thal LJ, Gage FH, Lee KF. (2003) Aberrant patterning of neuromuscular synapses in choline acetyltransferase-deficient mice. J Neurosci 23(2):539-49.

D'Amour KA and Gage FH (2002). Are somatic stem cells pluripotent or lineage-restricted? Nat Med 8: 213-4.

D'Amour, K and Gage F.H. (2000) New tools for human developmental biology. Nature Biotechnol 18:381-382.

D'Amour, K.A. & Casida, J.E. (1999) Desnitroimidacloprid and nicotine binding site in rat recombinant α4β2 neuronal nicotinic acetylcholine receptor. Pesticide Biochem Physiol 64:55-61.

Latli, B., **D'Amour, K.**, Casida, J.E. (1999) Novel and potent 6-chloro-3-pyridinyl ligands for the α4β2 neuronal nicotinic acetylcholine receptor. J Med Chem 42(12):2227-2234.

Bobilya, D.J., **D'Amour, K.**, Palmer, A., Skeffington, C., Therrien, N., Tibaduiza, E.C. (1995) Isolation and cultivation of porcine brain capillary endothelial cells as an in vitro model of the blood-brain barrier. Meth Cell Sci 17(1):25-32.

7111617:050809